Frequency of virus-resistant hosts determines experimental community dynamics.

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Title: Frequency of virus-resistant hosts determines experimental community dynamics

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Abstract

Parasites, such as bacterial viruses (phages), can have large effects on host populations both at the ecological and evolutionary levels. In the case of cyanobacteria, phages can reduce primary production and infected hosts release intracellular nutrients influencing planktonic food web structure, community dynamics and biogeochemical cycles. Cyanophages may be of great importance in aquatic food webs during large cyanobacterial blooms unless the host population becomes resistant to phage infection. The consequences on plankton community dynamics of the evolution of phage resistance in bloom forming cyanobacterial populations is still poorly studied. Here we examined the effect of different frequencies of a phage-resistant genotype within a filamentous nitrogen-fixing *Nodularia spumigena* population on an experimental plankton community. Three *Nodularia* populations with different initial frequencies (0%, 5% and 50%) of phage-resistant genotypes were inoculated in separate treatments with the phage 2AV2, the green alga *Chlorella vulgaris* and the rotifer *Brachionus plicatilis* which formed the experimental plankton community subjected to either nitrogen-limited or nitrogen-rich conditions. We found that the frequency of the phage-resistant *Nodularia* genotype determined experimental community dynamics. Cyanobacterial populations with a high frequency (50%) of the phage-resistant genotype dominated the cultures despite the presence of phages, retaining most of the intracellular nitrogen in the plankton community. In contrast, populations with low frequencies (0% and 5%) of the phage-resistant genotype were lysed and reduced to extinction by the phage, transferring the intracellular nitrogen held by *Nodularia* to *Chlorella* and rotifers, and allowing *Chlorella* to dominate the communities and rotifers to survive. This study shows that even though phages represent minuscule biomass, they can have key effects on community composition and eco-evolutionary feedbacks in plankton communities.

Keywords: experimental evolution, eco-evolutionary feedbacks, phage resistance, cyanobacteria, host-parasite interaction, predator-prey interaction

INTRODUCTION

Consumer-resource interactions represent one of the key building blocks in virtually any ecological community. In plankton food webs, the most studied consumer resource interactions has been the one between the phytoplankton and their zooplankton grazers. However the important role of viral parasites has been increasingly acknowledged during recent decades (Suttle, 1994; Weinbauer *et al.*, 2003; Brussaard, 2004; Suttle 2007; Wilhelm and Matteson, 2008; Wirington et al., 2016). Viruses can alter community composition, element cycling and be the major cause of the mortality in the host populations (Weitz, 2016). Furthermore, viral lysis can have larger scale effect beyond direct effects on their hosts by releasing cellular material back to the microbial loop which in turn can have effects on higher trophic levels (Fuhrman, 1999; Weitz *et al*., 2015; Weitz, 2016). At the same time, growing number of studies has examined how rapid, contemporary evolution can change the ecological dynamics (Yoshida *et al.*, 2003; Hiltunen and Becks, 2014; Koch *et al.*, 2014; Frickel *et al.*, 2016). In the case of microbial host-virus systems the ecological effects of the viruses are not constant since the host resistance can evolve very rapidly (Buckling and Rainey, 2002). Resistance evolution and the subsequent alternation of the ecological interaction can radically change role of viral lysis on food webs. However little or no information exists on how rapid evolution in host resistance

effects planktonic communities especially when the key community member is a nitrogen-fixer, a property that can have large indirect effects on the whole community by modulating the transfer of nutrients.

In our study, we used an experimental model system of a simple aquatic community in which viral resistance by nitrogen-fixing cyanobacteria forms a decisive component. The focus of our study was to investigate how host resistance evolution affects the ecological effect of virus infection on community dynamics, which in return can be important for our understanding how cyanobacterial blooms are formed. A large body of research has focused on the abiotic conditions leading to bloom formation and termination (Kanoshina *et al.*, 2003; Paerl and Huisman 2008). Moreover, biotic factors including parasites and grazers such as phages and zooplankton can exert top-down control on cyanobacterial populations (Brussaard *et al.*, 2008; Lemaire *et al.,* 2012; Storesund *et al.*, 2015). Since many cyanobacterial species are toxic or low-quality food for zooplankton (Sarnelle, 2007), zooplankton predators may preferentially graze on other primary producers, facilitating cyanobacterial bloom formation (Mitra and Flynn, 2006; Gorokhova and Engström-Öst, 2009). Compared to grazers, host-specific phages may exert greater selective pressure, causing selection for phage resistance and altering the genetic diversity of host populations (Winter *et al.*, 2004; Clokie *et al.*, 2011). The evolution of phage resistance allows for the emergence of different genotypes that can affect ecological interactions at the population level, in turn, influencing community structure and dynamics (Bohannan and Lenski, 2000). However, phages are often not incorporated in models on the transfer of energy pathways and fluxes (in classical or microbial food webs), even though they may have important implications for biogeochemical cycles (Suttle, 2007). Evolving interactions between cyanobacteria and cyanophages have received substantial attention during the past decade,

93 although the focus has primarily been on unicellular cyanobacteria rather than morphologically
94 more complex filamentous forms (Marston, 2012; Dekel-Bird *et al.*, 2013; Martiny *et al.*, 2014;
95 Avrani and Lindell, 2015). Recently, we demonstrated that phage resistance evolution can alter
96 phage-mediated nitrogen release in filamentous cyanobacterial populations but potential larger,
97 community-level effects remain unclear (Cairns *et al.*, 2016; Coloma *et al.*, 2017).
98 Cyanobacteria, similar to other primary producers, transfer atmospheric carbon and
99 nitrogen to higher trophic levels in the planktonic food web, playing an important role in aquatic
100 biogeochemical cycles (Richardson and Jackson, 2007; Ploug *et al.*, 2010). Understanding
101 nitrogen fluxes in the planktonic food web is highly important when nitrogen is limiting primary
102 production, as in most marine systems including vast regions of the Baltic Sea (Granéli *et al.*,
103 1990). Furthermore, how phage infection affects this process is largely unknown (see however
104 Cairns *et al.*, 2016; Coloma *et al.*, 2017; Shelford and Suttle, 2017). Under nitrogen limiting
105 conditions, nitrogen-fixing cyanobacteria can have a competitive advantage over other primary
106 producers due to their unique capability to fix dissolved gaseous nitrogen (Tamminen and
107 Andersen, 2007). Nitrogen-fixing cyanobacteria may exudate even 50% of the recently fixed
108 nitrogen (mainly as ammonium) enriching the dissolved nitrogen pool (Karl *et al.*, 1992;
109 Mulholland *et al.*, 2006). This increases the availability of nitrogen for other phytoplankton
110 species. Therefore, seasonal blooms of nitrogen fixing cyanobacteria can be an important source
111 of nitrogen (Kozlowsky-Suzuki *et al.*, 2007; Adam *et al.*, 2016), in particular, if phage-induced
112 cell lysis enhances the rate of nitrogen release, redirecting the intracellular nitrogen through a
113 process known as the viral shunt (Wilhelm and Suttle, 1999). Thus, phages can play a key role in
114 nutrient cycling and resulting ecosystem dynamics (Glibert and Bronk, 1994; Weitz and
115 Wilhelm, 2012; Coloma *et al.*, 2017). In this study, we used a microcosm approach to investigate

the influence of phage infection and phage resistance evolution in the host (*Nodularia spumigena*) on experimental plankton community dynamics. The members of our experimental community included a cyanobacterium (*Nodularia spumigena*), green alga (*Chlorella vulgaris*), herbivorous zooplankton (rotifer: *Brachionus plicatilis*) and *Nodularia*-infecting phage (vB_NpeS-2AV2) (Fig. 1).We manipulated the initial frequencies (i.e. the initial evolutionary state of the community) of the phage-resistant and susceptible genotypes of *Nodularia* and the availability of nitrogen in the culture media. The different initial frequencies of the genotypes were expected to determine different genotype-level trajectories of *Nodularia* populations under phage infection, and nitrogen availability in the medium y by altering the role of nitrogen fixation by *Nodularia* as a nitrogen source. Our prediction was that the presence of a phage-resistant genotype would lead to dominance of the resistant genotype and *Nodularia* dominance at the community level. The experimental outcome supported the prediction, indicating that when the initial *Nodularia* population included 50% of the phage-resistant genotype, the phytoplankton community was dominated by *Nodularia*, whereas green algae dominated the community when starting with 0% or 5% of the phage-resistant *Nodularia* genotype.

METHODS

Study species and culture conditions

The experimental plankton community was composed of primary producers (cyanobacteria and green algae), a parasite (phage) and zooplankton grazer. The primary producers were two species of photoautotrophs: the filamentous cyanobacterium *Nodularia spumigena* and the green

alga *Chlorella vulgaris,* hereafter referred as *Nodularia* and *Chlorella*. Non-axenic cultures of the filamentous nitrogen-fixing cyanobacteria *Nodularia spumigena* strain UHCC 0040 were obtained from the Cyanobacterial Collection HAMBI/UHCC (Sivonen *et al.*, 1989), University of Helsinki, Finland. The single-celled green alga *Chlorella vulgaris* strain UTEX 26 was obtained from the Culture Collection of Algae, Texas University (Austin, Texas, U.S.A). Both strains had been successfully cultured under similar conditions previously (Cairns *et al.*, 2016; Coloma *et al.*, 2017). *Nodularia* and *Chlorella* were cultured separately in liquid medium before inoculation in the microcosm experiment. *Chlorella* was cultured in Z8SN, a medium containing nitrogen (906 µM of N) and all other nutrients in non-limiting concentrations (Kotai, 1972), and *Nodularia* in Z8S, a medium containing the same nutrients but without nitrogen (Lehtimäki *et al.*, 1994). The Z8SN and Z8S culture media were prepared in type-2 analytical grade water (ELIX®, Merck Millipore, Billerica, MA, USA) in glass bottles and sterilized by autoclaving. Both strains were cultured at 21 ± 1°C with a 24 h continuous irradiance between 5–8 PPFD (µmol photons m^{-2} s^{-1}). Light intensity was measured with the LI-COR® LI-250 light meter (LO-CR, Lincoln, NE, USA).The parasite used for this study was the *Nodularia*-infecting lytic cyanosiphovirus vB_NpeS-2AV2 (hereafter, 2AV2) previously isolated from the Baltic Sea and stored at +4°C (Coloma *et al.*, 2017). Fresh virus stocks were prepared by infecting an exponentially growing culture of *Nodularia*. After cell lysis, the culture was centrifuged (7000 × *g*, 7 min at +4°C) and the supernatant stored at +4°C.

As a zooplankton grazer, we used the rotifer species *Brachionus plicatilis* from the Monogononta class (hereafter, rotifer). Rotifer resting eggs were obtained from Florida Agua Farms Resting Inc. (Dade City, Florida, USA). Prior to the experiment, eggs were hatched in cell culture bottles placed in continuous light in Z8SN medium enriched with vitamin B12, since

vitamin B12 enhances rotifer growth (Scott, 2009). Rotifer cultures were fed with *Chlorella*, grown on vitamin B12 enriched medium (Hirayama *et al.*, 1989). For the microcosm experiment, vitamin B12 was added to the initial medium and to the weekly replaced medium to avoid inhibition of rotifer growth. Rotifers were collected by filtering the cultures on a 40 µm net and rinsing them afterwards with Z8S medium.

Experimental setup and sampling

A microcosm experiment was used to observe interactions between the selected organisms. The experiment consisted of semi-continuous cultures, which were examined for 20 weeks and contained all organisms: *Nodularia*, *Chlorella*, phage 2AV2 and rotifers. The treatments consisted of three different frequencies of a phage-resistant *Nodularia* genotype: 0%, 5% or 50% of resistant genotype (evolved clones) with 100%, 95% and 50% of susceptible genotype (naive clones), respectively. Evolved clones were obtained from a previous experiment where phage-resistant filaments had been isolated (Cairns *et al.*, 2016). The three different treatments were cultured in a medium without added nitrogen (referred to as N-lim) and with nitrogen (referred to as N-rich, containing 400 µM of N as $NaNO_3$). We chose the concentration for the N-rich medium based on previous studies with rotifer-algal systems using 514 µM of N for elevated nutrient conditions (Fussmann *et al.*, 2000; Yoshida *et al.*, 2003). We kept the concentration slightly lower to avoid problems arising from light limitation. Overall, the experiment consisted of six different treatments that were replicated four times, distributed in 24 batch culture flasks with 500 ml of medium containing the study organisms (with the exception of three replicates in the N-lim medium treatments with 0% of the phage-resistant *Nodularia* genotype).

From each culture flask, 50 ml samples were collected at 7 day intervals. The removed 50 ml volume was replaced with the same volume of fresh culture medium immediately after sampling, corresponding to a dilution rate of 10% per week. For data analysis, we collected samples at 2 week intervals from week 2 to 8, and two later points represented by week 12 and 20. Samples from each time point were divided into sub-samples in order to count *Nodularia*, *Chlorella* and rotifer densities. Plaque-based assays were performed to determine the number of infective phage particles as plaque forming units (PFU) which was used as a measure of phage quantity. For this purpose, samples of 1 ml were stored in dark at +4°C for later PFU quantification.

Determining population sizes

Phytoplankton—Nodularia samples were fixed with Lugol's solution and *Chlorella* samples with glutaraldehyde solution (both at 2% final concentration), and stored in dark at +4°C. For cell counting, images of *Nodularia* samples were taken with an Olympus SC30 digital camera connected to a CKX41 Olympus inverted microscope with a 4× objective. Cyanobacterial filaments length was measured using the CellSens standard (version 1.7, Olympus) software. The filament length was divided by the average cell size to obtain final cell density. *Chlorella* samples were first pipetted through a 40 µm net (Corning® 40µm Cell Strainer) to remove rotifers and most of the filamentous cyanobacteria, avoiding the interference of large organisms with cell counting. From filtered samples, two technical replicates of 10 µl were pipetted to an improved Neubauer counting chamber with 0.01 mm depth (Marienfeld, Germany). *Chlorella* cells were then counted using the epifluorescence Carl Zeiss Axioskop 2 plus microscope with a 40× objective, TRITC fluorescence filter and HBO 100 W mercury vapor short-arc lamp.

Phage—Phage numbers were determined by plaque assay. For this purpose, 100 µl of a previously diluted phage sample was mixed with 1 ml of the host culture (*Nodularia*) and 3 ml of 0.25% soft agarose in Z8S medium. The mixture was poured over a plate with a bottom layer containing 0.5% agarose in Z8S medium and left to cool down at room temperature. The two-layer plates were covered with punctured parafilm to reduce water evaporation but permitting the exchange of gases. Culturing was performed at 25 ± 2°C at a continuous light intensity of 5–8 µmol m^{-2} s^{-1}. The number of phage particles was determined by counting the number of PFUs formed on seeded agarose plates. We therefore only considered the number of infective phage particles in this study.

Rotifers—Our rotifers can reproduce asexually through parthenogenesis and have a short juvenile period. For this reason we count female and juvenile abundance as a close proxy for rotifer abundance. Here, females were counted to represent rotifer abundance. Females were counted immediately after sample collection from three technical replicates of 0.5 or 1 ml drops using the Leica WILD M10 microscope.

Estimation of nitrogen content and threshold food concentration for rotifers

The nitrogen content was determined experimentally for *Nodularia* and *Chlorella* estimated based on literature for the phage and rotifer. The nitrogen content was used to observe the share of intracellular nitrogen between the study organisms. To estimate intracellular nitrogen content, *Chlorella* was cultured in N-lim and N-rich (80 µM of N) medium and *Nodularia* in N-lim and N-rich (400 µM of N) medium. To avoid light limitation *Chlorella* was cultured in a lower nitrogen concentration as previously in rotifer-algal chemostat systems (Becks *et al.*, 2010).

Aliquots of *Chlorella* and *Nodularia* suspensions were filtered onto 25 mm precombusted glass fiber filters (GF⁄F, Whatman, Dassel, Germany), dried at 50 °C and analyzed using an elemental analyzer (Euro EA 3000, HEKAtech GmbH, Wegberg, Germany). The intracellular nitrogen content of *Chlorella* measured in N-lim medium after 6 days was $6.4 \times 10^{-5} \pm 0.5 \times 10^{-5}$ ng N cell^{-1} (average ± S.E., N = 3) and in N-rich medium (80 µM of N) after 5 days $5.1 \times 10^{-4} \pm 0.7$ 10^{-4} ng N cell^{-1} (N = 3). These values were considered as the minimum and maximum nitrogen content for *Chlorella*, and the mean value was used for statistical analysis. The intracellular nitrogen content of *Nodularia* measured in N-lim medium was $3.0\ 10^{-3} \pm 0.4\ 10^{-3}$ ng N cell^{-1} and in N-rich medium (400 µM of N) $2.2 \times 10^{-3} \pm 0.5 \times 10^{-3}$ ng N cell^{-1}. The nitrogen content considered for an individual eggless adult rotifer was 28.5 ng N individual^{-1} (Nagata, 1989). Makridis and Olsen (1999) and Schlosser and Anger (1982) found similar nitrogen contents in *B. plicatilis* adults. According to Nandini *et al.* (2007), the minimum *Chlorella* density for maintaining growth of *Brachionus* species is 0.1×10^6 cell ml^{-1} which was used as the threshold food concentration. The nitrogen content of the phage 2AV2 was estimated to be comparable to the morphologically similar phage T4 (Jover *et al.*, 2014) and therefore 6.1×10^{-8} ng virus particle^{-1} (based on genome length).

Statistical analyses—Repeated Measures ANOVA (RMANOVA) was used to compare *Nodularia*, phage, *Chlorella* (average values) and rotifer densities between treatments with 0%, 5% and 50% of phage-resistant *Nodularia* genotype, and between N-lim and N-rich conditions. Multiple comparisons were performed using Tukey's range test. RMANOVA analyses were performed with SPSS Statistics (IBM SPSS Statistics, version 22).

253 RESULTS

254

255 *Influence of phage resistance on community dynamics*

256 The dynamics of the different plankton groups were compared between treatments with different
257 frequencies of the phage-resistant genotype in N-lim and N-rich medium. The statistical analysis
258 showed a lack of significant difference between N-lim and N-rich medium conditions for
259 *Nodularia*, phage 2AV2 and *Chlorella* densities ($F_{1,21} = 0.1$, $P > 0.05$, $F_{1,21} = 0.7$, $P > 0.05$, and
260 $F_{1,21} = 1.8$, $P > 0.05$, respectively; Table 1). In contrast, rotifer densities differed significantly
261 between N-lim and N-rich medium conditions ($F_{1,21} = 13.1$, $P < 0.05$). Furthermore, *Nodularia*,
262 phage and *Chlorella* densities differed significantly between treatments with different
263 frequencies of phage-resistant *Nodularia* genotype in N-lim and in N-rich conditions (*Nodularia*;
264 $F_{2,8} = 407.2$ and $F_{2,9} = 69.3$, phage; $F_{2,8} = 153.1$ and $F_{2,9} = 87.5$, and *Chlorella*; $F_{2,8} = 38.5$ and
265 $F_{2,9} = 170.0$, respectively and all $P < 0.05$; Table 2). For rotifers, this holds only for N-rich
266 medium and not for N-lim medium conditions ($F_{2,9} = 317.9$, $P < 0.05$ and $F_{2,8} = 1.5$, $P > 0.05$,
267 respectively).

268 *Nodularia* densities were higher in cultures with a high frequency of the phage-resistant
269 genotype (initially 50%) compared to cultures with low frequencies (0% and 5%) in both
270 nitrogen conditions (Fig. 2AB). In cultures with low phage-resistant genotype frequencies (0%
271 and 5%), *Nodularia* densities decreased until extinction under both nitrogen conditions (Fig.
272 2AB). This occurred earlier in the presence of 0% compared to 5% of the phage-resistant
273 genotype. Phage and *Chlorella* densities exhibited the opposite pattern to *Nodularia* (Fig. 2CD
274 and 2EF), showing higher densities in cultures with low phage-resistant frequencies (0% and
275 5%) and lower densities in cultures with high phage-resistant genotype frequencies (50%).

276 *Chlorella* densities increased towards the end of the experiment in cultures with 0% and 5% of
277 phage-resistant *Nodularia* genotype in N-rich medium in contrast with the more stable densities
278 in N-lim medium (Fig. 2EF). In addition, approximately 5 times higher rotifer densities were
279 observed in N-rich conditions with low frequency of phage-resistant *Nodularia* genotype (Fig.
280 2H) compared to N-lim conditions (Fig. 2G). In treatments with 50% of phage-resistant
281 *Nodularia* genotype, *Chlorella* densities dropped under the minimum level (0.1×10^6 cell ml^{-1},
282 see Methods) needed to maintain the rotifer population in week 20 in both medium conditions. In
283 line with the persistently low *Chlorella* densities since week 5, rotifer densities decreased until
284 extinction in both medium conditions at week 20 (Fig. 2GH).
285
286 *Plankton succession and estimated nitrogen transfer in the plankton community*
287 Plankton community dynamics and nitrogen transfer in the plankton food web were examined by
288 comparing the relative share of *Nodularia*, *Chlorella* and rotifers to the sum of the estimated
289 intracellular nitrogen concentration (ng N ml^{-1}). The estimated intracellular nitrogen content was
290 based on separate measurements for the phytoplankton species and on literature values for the
291 phage and rotifer. Because the level of intracellular nitrogen may vary over time depending on
292 growth conditions, these values provide only a rough estimate of the nitrogen content. Initially,
293 the *Nodularia* population held between 69% and 79% of the plankton community nitrogen in all
294 treatments (Fig. 3A–F). During the experiment, nitrogen was transferred gradually from
295 *Nodularia* to *Chlorella* and rotifer populations in cultures with low frequencies (0% and 5%) of
296 the phage-resistant *Nodularia* genotype (Fig. 3A–D). At the end of the experiment in week 20,
297 *Chlorella* dominated and held between 93% and 99% of the nitrogen in the plankton community
298 in these treatments. In addition, in treatments with N-rich medium, rotifers held the majority of

299 the nitrogen in week 4: 81% and 77% in cultures with 0% and 5% of phage-resistant *Nodularia*
300 genotype, respectively (Fig. 3BD). In cultures with 50% of phage-resistant *Nodularia* genotype,
301 the *Nodularia* population held the majority of the intracellular nitrogen throughout the
302 experiment, increasing from 78% to 99% in N-lim and 73% to 99% in N-rich medium conditions
303 toward the end of the experiment (Fig. 3EF). The *Nodularia* population reached the highest
304 biomass in week 20 with $3.1 \times 10^4 \pm 0.3 \times 10^4$ and $3.4 \times 10^4 \pm 0.4 \times 10^4$ ng N ml^{-1} in N-lim and
305 N-rich medium, respectively. Comparatively less nitrogen was contained in the *Chlorella*
306 population when *Chlorella* dominated, ranging between $8.4 \times 10^2 \pm 6.6 \times 10^2$ and $5.6 \times 10^3 \pm 4.3$
307 $\times 10^3$ ng N ml^{-1} (Fig. 3A and 3D respectively) on week 20. The relative share of intracellular
308 nitrogen between the experimental plankton components assuming the maximum and minimum
309 *Chlorella* nitrogen content is shown in Fig. S1 and S2, revealing same dominance outcome at the
310 end of the experiment. Overall, *Nodularia* maintained dominance in cultures with high frequency
311 (50%) of the phage-resistant genotype, and *Chlorella* became dominant in cultures with low
312 frequencies (0% and 5%) of the phage-resistant *Nodularia* genotype. Consequently, the
313 succession caused by low phage-resistant genotype frequencies potentially affected the nitrogen
314 transfer our experimental system.
315 The relationship between the share of *Chlorella* in the total phytoplankton density and
316 rotifer abundances reveals predator-prey interactions (Fig. S3). The initially increasing share of
317 *Chlorella* declined after the rotifer peak in cultures with low frequencies (0% and 5%) of the
318 phage-resistant *Nodularia* genotype under N-lim medium conditions (Fig. S3A–D), although
319 increasing afterwards reaching 100% of the phytoplankton (weeks 12 and 20). In cultures with
320 N-rich medium, the rotifer peak had a lower effect on the *Chlorella* share (Fig. S3BD) that

reached the 100% of the phytoplankton earlier than in N-lim medium conditions (weeks 6 and 12).

In cultures with an initially high frequency (50%) of the phage-resistant *Nodularia* genotype, the contribution of *Chlorella* decreased constantly, remaining low throughout the experiment (Fig. S3EF). Despite the lower contribution of *Chlorella*, the rotifer densities reached a peak similar to cultures with low frequencies (0% and 5%) of the phage-resistant *Nodularia* genotype in week 4 (Fig. S3EF). The rotifer density peak remained much lower in cultures with a high frequency (50%) of the phage-resistant *Nodularia* genotype with N-rich medium compared to all other treatments. Notably, the formation of *Chlorella* colonies (cell clumping) inedible to the rotifers was detected during the experiment in all treatments (Fig. S4).

DISCUSSION

The two key determinants of prey community composition and dynamics are competition for shared resources and predation, including by parasites. The effects of predation and parasitism are widely studied, and consumer-mediated coexistence is a highly important and classic notion in ecology. However, little is known about how rapid evolutionary changes in key traits, such as resistance against parasites, contribute to species co-existence and overall community dynamics (see however Hiltunen *et al.*, 2014 and Frickel *et al.*, 2017). For instance, if the relative role of predation or parasitism is reduced due to resistance evolution, community dominance might shift toward species that are resistant but competitively inferior in the absence of the consumers. In general, the evolution of phage resistance is known to affect competitive traits in the bacterial host such as causing reduced growth rate (Bohannan and Lenski., 2000; Avrani *et al.*, 2011). In

cyanobacteria, phages reduce the number of susceptible cells and select for phage-resistant cells (Šulčius *et al.*, 2015). In a previous study (Cairns *et al.*, 2016), we tried to identify potential costs of resistance by measuring the growth of susceptible and resistant genotypes in different conditions but we did not observe any differences. Low resistance costs could enhance the survival of the resistant genotype and ultimately promote the phage extinction.

Here our aim was to clarify the community wide impact of resistance evolution in a complex community involving prey competition, predation and parasitism. We investigated the dynamics of an experimental plankton community with two competing primary producers (a cyanobacterium, *Nodularia* and a green alga, *Chlorella*), a specialist consumer (phage parasite) and a generalist consumer (rotifer predator). We manipulated the initial frequencies of phage-resistant and susceptible cyanobacterial genotypes. We found that the initial frequencies of the genotypes at the onset of the experiment determined planktonic community dynamics such that cultures with a high frequency (50%) of the phage-resistant *Nodularia* genotype led to a dominance of *Nodularia*, and cultures with low frequencies (0% and 5%) were *Chlorella*-dominated also facilitating the persistence of the generalist grazer (rotifers). Qualitatively, a similar community dynamics pattern was observed regardless of the concentration of added nitrogen, although the densities of *Chlorella* and rotifer populations were higher when nitrogen was added to the culture medium.

In general, phage-mediated host mortality can regulate phytoplankton dynamics and diversity, affecting community dynamics (see review by Brussaard, 2004). In the case of nutrient fluxes, through the viral shunt, phage-mediated redirection of intracellular nutrients incorporated in cyanobacteria can rapidly increase the amount of available nitrogen in the surrounding aquatic environment (Wilhelm and Suttle, 1999). We observed this pattern in treatments with a low

frequency of the phage-resistant genotype where phage-mediated host mortality directly affected *Nodularia* and indirectly the other community members. Moreover, our results indicate that the phage-mediated nitrogen release can facilitate the growth of competitors, providing a double advantage to competitors of *Nodularia* under phage infection. Here, susceptible *Nodularia* was suppressed by phage infections giving a competitive advantage to *Chlorella* which is not infected by the phage. *Chlorella* also likely benefitted directly from the lysis of *Nodularia* as the nitrogen contained in *Nodularia* cells was released and could have been used by *Chlorella* (Cairns *et al.*, 2016). Initially, the increased availability of nitrogen promoted the increase of *Chlorella* and rotifer populations in our experiments. Although rotifers had only one initial peak, they remained at stable densities hindering the growth of *Chlorella*. The evolution of defence against grazing by *Chlorella* is one potential reason why rotifer abundances declined over time even at high *Chlorella* biomasses (Fig. S3). *Chlorella* may evolve an effective heritable defence against grazers, forming stable colonies of multicellular *Chlorella* that rotifers cannot feed on effectively (Yoshida *et al.*, 2003; Yoshida *et al.*, 2004).

A 50% initial frequency of the phage-resistant *Nodularia* genotype was enough to allow the *Nodularia* population to grow and dominate the planktonic community despite the presence of *Nodularia*-infecting phages. One explanation for cyanobacterial blooms is that their ability to fix nitrogen gives them a competitive advantage over other algae under nitrogen-limited conditions. This likely contributed to the dominance of the phage-resistant *Nodularia* population over *Chlorella*. Our results indicate that the ratio between the phage-resistant and susceptible genotypes may be one key biotic aspect influencing the development of cyanobacterial blooms.

The toxicity or superiority in resource competition of *Nodularia* may explain the decrease in the *Chlorella* population regardless of the input of new nitrogen leaked from the dominant

390 nitrogen-fixing cyanobacteria. After *Chlorella* densities dropped under the threshold
391 concentration, rotifer densities decreased until extinction despite the fact that *Brachionus* species
392 have been found to feed on filamentous cyanobacteria by nibbling at filament ends in the
393 absence of more suitable food sources (Dumont, 1977). Potential reasons why *Nodularia* alone
394 cannot sustain rotifer growth include toxicity, mechanical interference and the low nutritional
395 value of cyanobacteria (Porter and Orcutt, 1980; Gulati and DeMott, 1997). Furthermore, the
396 ratio between low quality or toxic food and high quality food can be important in determining
397 grazer growth (Hiltunen *et al.*, 2012). In line with this, higher *Chlorella* biomasses supported
398 higher rotifer biomasses in cultures with 0% and 5% of the phage-resistant *Nodularia* genotype
399 in both medium conditions. In addition, one possible scenario observed in many bacteria-phage
400 is studies is the co-evolution of the phage (e.g. Buckling and Rainey, 2002; Paterson *et al.*,
401 2010). This possibility could lead to interesting longer-term dynamics. However, in earlier
402 studies with the same *Nodularia* strain and with comparable microcosm setups and time scales
403 (Cairns *et al.*, 2016; Coloma *et al.*, 2017), we did not find any evidence of co-evolution
404 indicating that co-evolution does not play a significant role in our set up.
405 In summary, our study demonstrates that phages, even though representing minuscule
406 biomass, can have a key effect on community composition and eco-evolutionary feedbacks. Our
407 study shows that the initial frequency of phage-resistant cyanobacterial genotypes is critical for
408 community dynamics, the succession of phytoplankton species and the transfer of nutrients
409 among plankton components, thereby indirectly affecting the entire food web. We also
410 hypothesise that phages of nitrogen fixing cyanobacteria can be keystone components in aquatic
411 food webs due to their capacity to release the nitrogen bound to cyanobacterial cells at short time
412 scales. The fact that other phototrophic members of the plankton community can use this

nitrogen makes the effect even larger. Interestingly, we also observe a community wide, indirect link between ecology and evolution. The effect of phages can be completely reversed if phage-resistant host genotypes are sufficiently abundant, highlighting the importance of understanding eco-evolutionary feedbacks in planktonic community dynamics.

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617 Table 1. Comparison between the growth of organisms in N-lim and N-rich medium (repeated

618 measures ANOVA)

Community component	*F*	*p*
Nodularia	0.097	0.758
Phage 2AV2	0.667	0.423
Chlorella	1.79	0.195
Rotifer	13.072	<0.05

619 Degree of freedom = 1; Degree of freedom (error) = 21.

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Table 2. Comparison between the growth of organisms with different frequencies of phage-resistant *Nodularia* genotypes for N-lim and N-rich treatments (repeated measures ANOVA)

Organism	Treatment	*F*	*p*
Nodularia	N-lim[a]	407.235	<0.05
	N-rich[b]	69.343	<0.05
Phage	N-lim[a]	153.055	<0.05
	N-rich[b]	87.461	<0.05
Chlorella	N-lim[a]	38.506	<0.05
	N-rich[b]	169.972	<0.05
Rotifer	N-lim[a]	1.461	0.288
	N-rich[b]	317.957	<0.05

Degree of freedom = 2; [a]Degree of freedom (error) = 8; [b]Degree of freedom (error) = 9.

FIGURE LEGENDS:

Figure 1. Experimental planktonic community composition and theoretical nitrogen pathways in the food web. Community members: (A) *Nodularia*-specific phage 2AV2, (B) *Nodularia spumigena*, (C) Heterotrophic bacteria, (D) *Chlorella vulgaris*, (E) *Brachionus plicatilis*. Arrows show hypothesised nitrogen (energy) pathways between community components, the nitrogen pool (DN = dissolved nitrogen) and (F) gaseous nitrogen.

Figure 2. Mean densities of plankton groups during the experiment: *Nodularia* (A–B), phages (C–D), *Chlorella* (E–F) and rotifer (G–H) in N-lim and N-rich medium. Dashed horizontal lines indicate rotifer food threshold, i.e. the estimated *Chlorella* biomass where rotifers maintain positive growth (E–F). Black (squares): densities in treatments with initially 0% of phage-resistant cyanobacteria; green (dots): densities in treatments with 5% of resistant cyanobacteria; red (triangle): densities in treatments with 50% of phage-resistant cyanobacteria. EXT = extinction, i.e. densities under the detection limit; ind. = individuals. Log error bars represent standard error from 4 replicates. Note the different scales of the y-axes.

Figure 3. Temporal changes in the relative contribution of different food web components to the sum of the estimated intracellular nitrogen content. Cultures with 0% (A–B), 5% (C–D) and 50% of phage-resistant *Nodularia* genotype (E–F), in N-lim and N-rich medium. The relative contribution of *Nodularia* (green), *Chlorella* (dark yellow), and rotifer (grey) populations are shown by bars (left y-axis scale) and the sum of the intracellular nitrogen content of the three food web components (ng N ml^{-1}) by a black line (right y-axis scale).

FIGURES

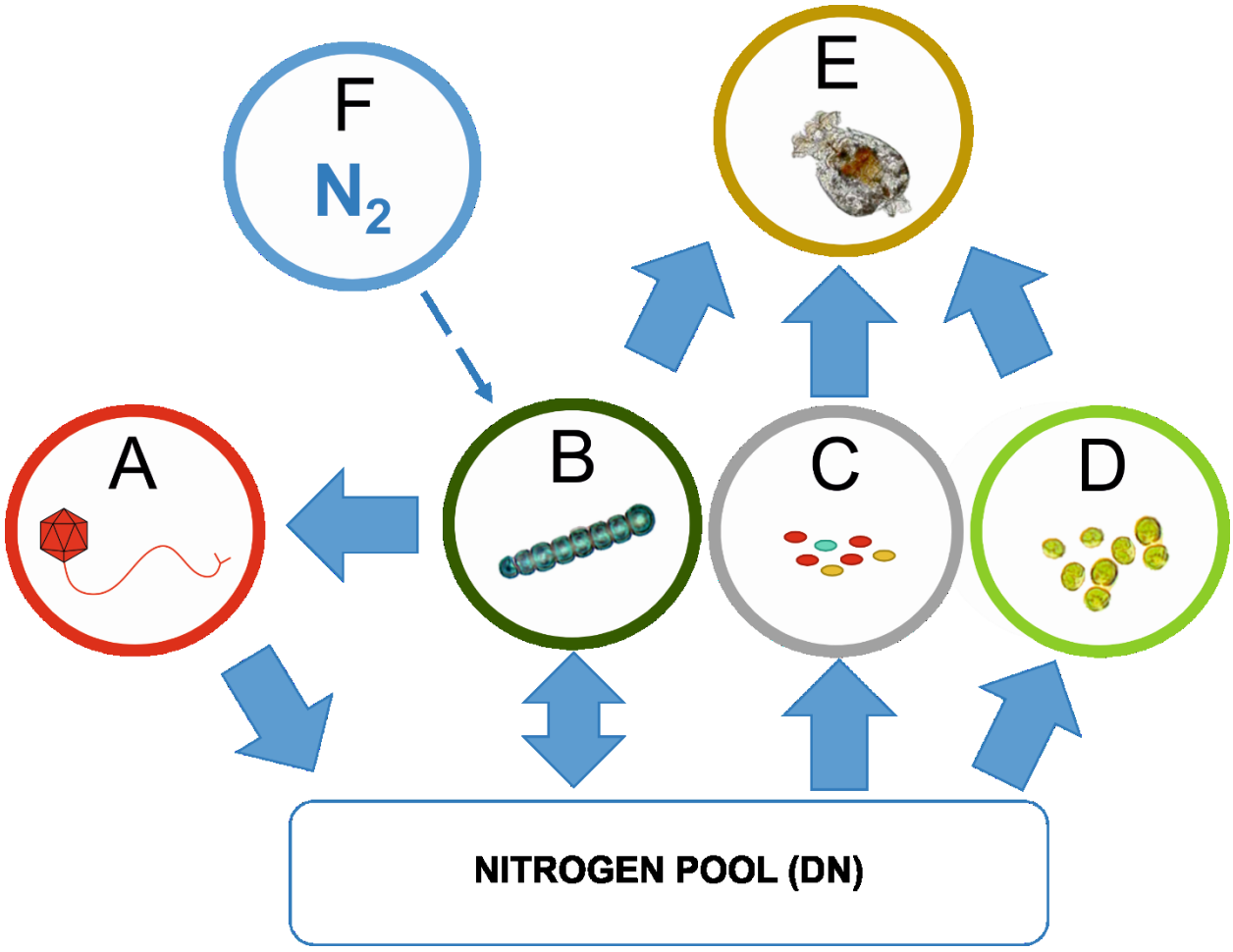

Figure 1

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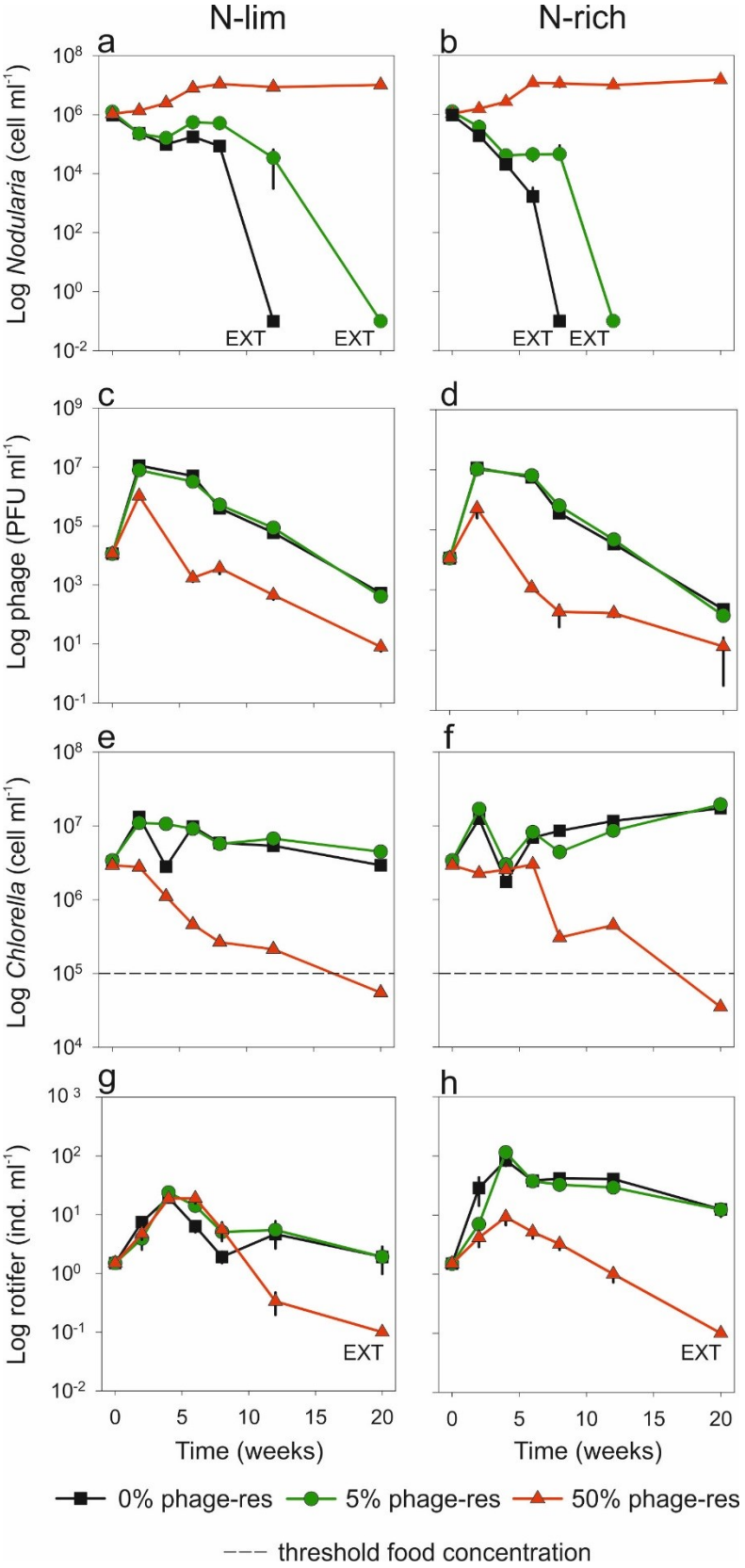

699 Figure 2

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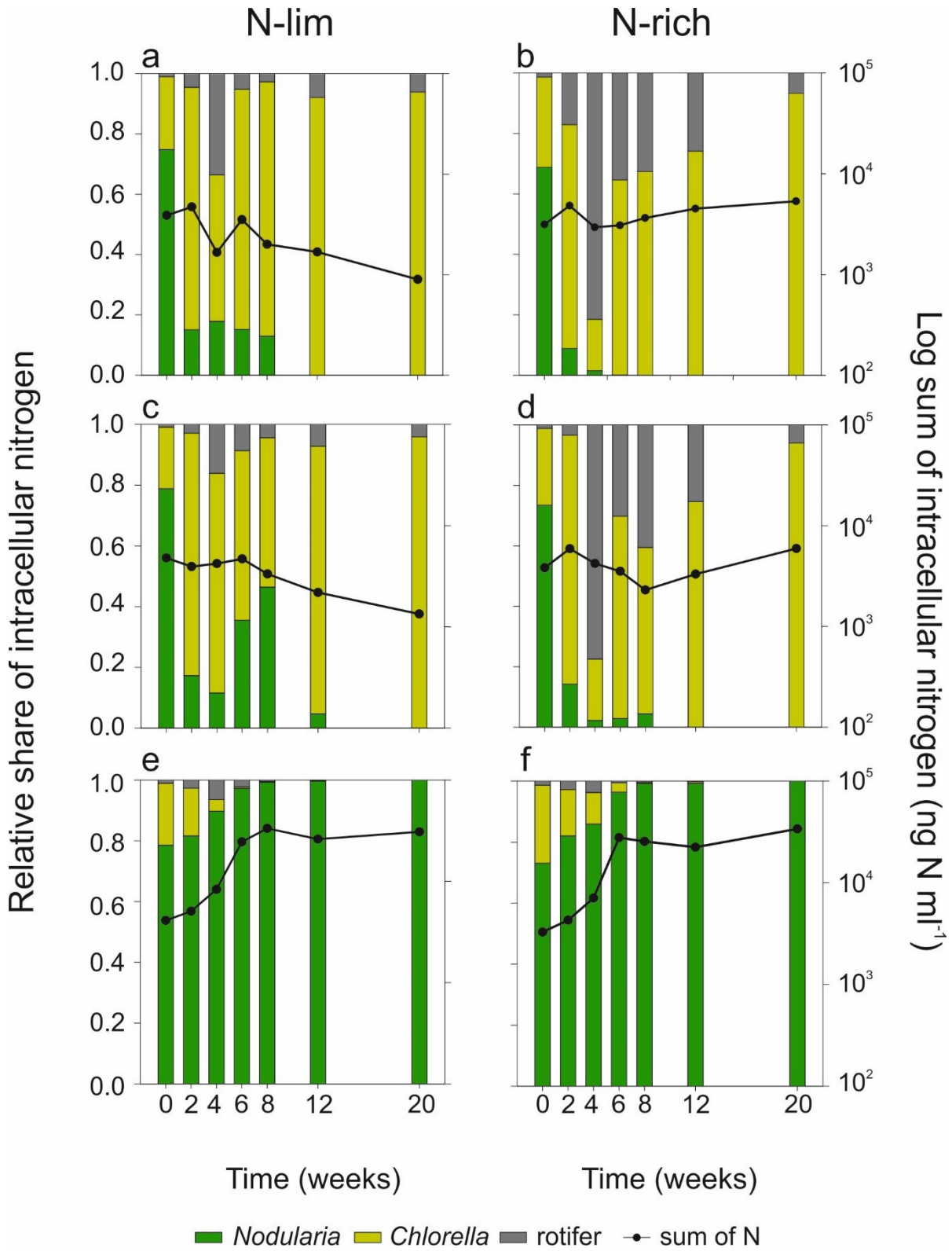

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702 Figure 3